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Relationship between Early Vasopressor Administration and Spinal Cord Hemorrhage in a Porcine Model of Acute Traumatic Spinal Cord Injury

Amanda Cheung,[1] Femke Streijger,[1,*] Kitty So,[1] Elena B. Okon,[1] Neda Manouchehri,[1] Katelyn Shortt,[1] Kyoung-Tae Kim,[1,2] Martin Sheung Man Keung,[1] Ryan M. Chan,[1] Allan Fong,[1] Jenny Sun,[1] Donald E. Griesdale,[3] Mypinder S. Sehkon,[4] and Brian K. Kwon[1,5]

Abstract

Current practice guidelines for acute spinal cord injury (SCI) recommend augmenting mean arterial blood pressure (MAP) for the first 7 days post-injury. After SCI, the cord may be compressed by the bone/ligaments of the spinal column, limiting regional spinal cord blood flow. Following surgical decompression, blood flow may be restored, and can potentially promote a ''reperfusion'' injury. The effects of MAP augmentation on the injured cord during the compressed and decompressed conditions have not been previously characterized. Here, we used our porcine model of SCI to examine the impact of MAP augmentation on blood flow, oxygenation, hydrostatic pressure, metabolism, and intraparenchymal (IP) hemorrhage within the compressed and then subsequently decompressed spinal cord.

Yucatan mini-pigs underwent a T10 contusion injury followed by 2 h of sustained compression. MAP augmentation of ~20 mm Hg was achieved with norepinephrine (NE). Animals received MAP augmentation either during the period of cord compression (CP), after decompression (DCP), or during both periods (CP-DCP). Probes to monitor spinal cord blood flow (SCBF), oxygenation, pressure, and metabolic responses were inserted into the cord parenchyma adjacent to the injury site to measure these responses. The cord was harvested for histological evaluation.

MAP augmentation increased SCBF and oxygenation in all groups. In the CP-DCP group, spinal cord pressure steadily increased and histological analysis showed significantly increased hemorrhage in the spinal cord at and near the injury site. MAP augmentation with vasopressors may improve blood flow and reduce ischemia in the injured cord but may also induce undesirable increases in IP pressure and hemorrhage.

Keywords: hemodynamics; MAP; pressure; spinal cord injury; vasopressor support

Introduction

There are currently limited treatment options for patients who suffer acute traumatic spinal cord injuries (SCI). Early treatment options aim to mitigate the secondary pathological processes, such as hypotension, decreased cord perfusion, and ischemia, that follow the primary mechanical impact.[1,2] The augmentation of mean arterial blood pressure (MAP) to increase spinal cord perfusion is one of the few treatment options available in clinical care that may improve neurological outcome.[3–6] Although clinical practice guidelines exist that recommend maintaining MAP at 85–90 mm Hg for 7 days post-injury, the guidelines themselves acknowledge that strong evidence linking such MAP augmentation to improved neurological outcome is lacking.[7]

Although it makes intuitive sense that MAP augmentation would improve spinal cord blood flow and thus reduce ischemic secondary injury to the spinal cord, the difficulty in establishing a strong relationship between MAP augmentation and improved neurological recovery in human SCI raises a number of considerations. First, MAP augmentation, which is typically achieved with vasopressors, may not have the expected effect on spinal cord blood flow (SCBF). A study using a rodent model of SCI induced by 1-min clip

[1]International Collaboration on Repair Discoveries, [5]Vancouver Spine Surgery Institute, Department of Orthopedics, The University of British Columbia, Vancouver, British Columbia, Canada.
[2]Department of Neurosurgery, Kyungpook National University Hospital, Kyungpook National University, Daegu, South Korea.
[3]Department of Anesthesiology, [4]Department of Medicine, Division of Critical Care Medicine, Vancouver General Hospital, The University of British Columbia, Vancouver, British Columbia, Canada.
*The first two authors contributed equally.

compression showed that MAP augmentation following injury did not improve SCBF at the injury site and additionally led to increased hemorrhage and edema.[8] MAP augmentation was associated with similar adverse effects in a feline model of contusive SCI, where elevating MAP resulted in worsened spinal cord hemorrhage and greater axonal damage.[9] Hence, the possibility that MAP augmentation does not actually achieve the intended neuroprotective effect needs to be considered.

Second, when MAP augmentation is instituted, the injured spinal cord itself may still be compressed, or it may be surgically decompressed. In rodent models of SCI, if sustained compression is applied (and in many cases it is not), the duration is typically quite short (e.g., 1 min), and subsequent MAP augmentation is performed in the decompressed state. However, in human SCI, the duration of compression may be very long, and in some patients the spinal cord is not even decompressed at all. In the Surgical Trial of Acute Spinal Cord Injury Study (STASCIS),[10] the average time between injury and surgical decompression in the ''early'' group was 14.2 ± 5.4 h, and it was 48.3 ± 29.3 h in the ''late'' group. This highlights the need to consider the effects of MAP augmentation in two distinct injury phases: 1) during sustained compression of the contused spinal cord, and 2) the period after decompression of the spinal cord (when compression has been removed).

In the present study, we aimed to determine how the augmentation of MAP in the presence or absence of spinal cord compression influenced the acute vascular and metabolic responses after traumatic SCI, using a large animal (pig) model of SCI. MAP augmentation was achieved with norepinephrine (NE), which we had observed in previous work provided better restoration of blood flow and oxygenation than phenylephrine.[11] The effects of MAP augmentation were assessed in the compressed and decompressed spinal cord. Specifically, we evaluated SCBF, oxygenation (in partial pressure of oxygenation; PO_2), spinal cord pressure (SCP), downstream metabolic responses, and hemorrhage within the spinal cord.

Methods

All animal protocols and procedures used in this study were approved by the Animal Care Committee of the University of British Columbia and were compliant with the policies of the Canadian Council of Animal Care and the U.S. Army Medical Research and Materiel Command (USAMRMC) Animal Care and Use Review Office (ACURO).

Animals and experimental groups

Female miniature Yucatan pigs (Sinclair BioResources, Columbia, MO, USA) weighing 21–30 kg arrived at the animal facility 5 weeks before surgery. Upon arrival, animals were housed in groups of two to four in an indoor pen bedded with sawdust and toys (chains and balls) with access to an adjoining outdoor pen. Animals were given water *ad libitum* and fed 1.5% of their body weight twice a day (Mazuri Mini-Pig Youth, PMI Nutrition International, Brentwood, MO, USA).

At the time of surgery, animals were randomized into four groups ($n = 6$/group): 1) no MAP augmentation (control group), 2) MAP augmentation during cord compression only (CP group), 3) MAP augmentation after cord decompression only (DCP group), and 4) MAP augmentation throughout cord compression and decompression (CP-DCP group) (Supplementary Fig. S1). All four groups received a T10 contusion injury and were evaluated for 2 h with sustained spinal cord compression and then another 2 h post-decompression (4 h total). To augment MAP $\sim$20 mm Hg from baseline, animals in the CP group received a 1.5 h infusion of NE (4 mg in 250 mL of 0.9% NaCl/1.25% dextrose) 30 min after SCI; the DCP group received a 1.5 h infusion of NE immediately after decompression; the CP-DCP group received a 3.0 h infusion of NE commenced 30 min after SCI. The control group did not receive MAP support and no NE was administered. Intravenous (IV) maintenance fluid rates (0.9% NaCl/1.25% dextrose) were uniquely adjusted for each animal to ensure a total fluid infusion of 7 mL/kg/h for all experimental groups during NE infusion.

Porcine model of SCI

Tracheal intubation, mechanical ventilation, and carotid artery and jugular vein catheterization to perform NE infusions were performed as previously described.[11] After catheterization, the animal was placed into the prone position and a skin incision was made along the dorsal midline of the thoracic region of each animal. Electrocautery (Surgitron® Dual Frequency RF/120 Device, Ellman International, Oceanside, NY, USA) was used to separate the semispinalis, multifidus, and longissimus lumborum muscles from the dorsal spinous processes, laminae, and transverse processes. A laminectomy at T9-13 was performed to expose the dura and spinal cord allowing sufficient space for sensor positioning and weight-drop injury.

The contusive SCI was induced by a weight-drop impactor device, which was securely fixed to the spine using an articulating arm (660, Starrett, Athol, MA, USA) mounted via bilaterally inserted T6 and T8 pedicle screws. This arm allowed the rail (which guided the impactor) to be precisely positioned and aligned, providing a straight vertical impact onto the exposed dura and cord at T10. The tip of the impactor (diameter: 0.953 cm) was equipped with a load cell (LLB215, Futek Advanced Sensor Technology, Irvine, CA, USA) to measure the force at impact. The guide rail was outfitted with a Balluff Micropulse Linear Position Sensor (BTL6-G500-M0102-PF-S115, Balluff Canada, Inc., Mississauga, Ontario, Canada) to record the impactor position from 10 cm above the impact (for calculation of impact velocity and cord displacement). A custom controller was used to operate the impactor device and filter the force, and position data was collected simultaneously with a USB DAQ Module (DT9816-S, Data Translation, Inc., Marlboro, MA, USA). Remote operation of the device and real-time data collection feedback were performed with the LabVIEW (National Instruments, Austin, TX, USA) program. Immediately after the weight-drop contusion injury (weight: 50 g; height: 20 cm), an additional 100-g weight was placed onto the impactor (150 g total) to sustain a 2 h compression period on the contused spinal cord.

Monitoring of intraparenchymal blood flow, partial pressure of oxygen (PO_2), pressure, and metabolic changes

Prior to SCI, a total of three intraparenchymal (IP) sensors were inserted through the dura into the spinal cord at T11 for the assessment of blood flow, PO_2 (NX-BF/OF/E, Oxford Optronix, Oxford, UK), hydrostatic pressure (FOP-LS-NS-1006A; FISO Technologies, Inc., Harvard Apparatus, Quebec, Canada), and metabolic changes (CMA11, CMA Microdialysis, Harvard Apparatus, Quebec, Canada). We employed a single multi-parameter probe for simultaneous measurement of SCBF and PO_2,[11] in which SCBF was measured using laser Doppler flowmetry[12] and PO_2 utilized a fluorescence quenching and fiberoptic technology.[13] SCP was monitored with a custom-made fiberoptic combined Fabry-Perot interferometry pressure sensor.[14,15] For sampling of metabolites in extracellular fluid, we utilized microdialysis probes with an outer diameter of 380 μm, a 2-mm membrane length, and a 6 kDa cutoff. A subcutaneous implantable micropump (SMP-200, IPrecio, Alzet Osmotic Pumps, Durect Corporation, Cupertino, CA, USA) was employed to continuously perfuse the probes with artificial cerebrospinal fluid (CSF) (Perfusion Fluid CNS, CMA Microdialysis, Harvard Apparatus, Quebec, Canada) at a flow rate of

0.5 μL/min. IP sensors were advanced at a 45-degree angle, to a cord depth of 4 mm at two locations along the cord, with the sensor tips approximately 2 mm and 22 mm caudal from the anticipated edge of the impactor.[16]

A custom-made platform, with precision-drilled holes, positioned above the cord and secured to the spine via 3.5-mm titanium rods (Medtronic) and pedicle screws (Medtronic) at T9, T11, T12, and T14 served as a guide to direct the placement of sensors into the cord.[16] Accurate sensor placements were verified by ultrasound imaging (L14-5/38, 38-mm linear array probe, Ultrasonix RP, BK Ultrasound, Richmond, British Columbia, Canada).

Data acquisition

IP blood flow and oxygen partial pressure sensors, connected to the OxyFlo/OxyLite Pro XL monitors (Oxford Optronix, Oxford, UK) recorded continuously at a sampling rate of 100 Hz and 1 Hz, respectively. IP pressure sensors were connected to a signal conditioner mounted on a multi-channel chassis (FISO Technologies, Inc., Harvard Apparatus, Quebec, Canada) and sampled at 100 Hz. All three parameters were collected via a PowerLab data acquisition device running LabChart version 7.3.8 (Colorado Springs, CO, USA). Microdialysis samples were collected every 15 min and analyzed using the IscusFlex Microdialysis Analyzer (M Dialysis, Stockholm, Sweden) for intracellular lactate (LAC), pyruvate (PYR), glucose (GLUC), glutamate (GLUT), and glycerol (GLY). The lactate to pyruvate (L/P) ratio was derived from the existing measurements of LAC and PYR concentrations. Baseline monitoring of all measurement parameters began 1 h before injury and data collection continued for 4 h post-injury. All values were expressed as the difference from baseline as a function of time (hours) to account for differences in absolute values from the baseline recordings.

Eriochrome Cyanine histochemistry

Spinal cord segments (3 cm) were collected and fixed in 4% paraformaldehyde (PFA), then cryoprotected using graded concentrations of sucrose (12, 18, 24, and 30% sucrose in 0.1 M phosphate buffer each for 48 h) and frozen in Tissue-Teck O.C.T. (Sakura Finetek USA, Inc.) at −80°C. Tissue was then transversely cut in 20-μm sections using a cryostat and Eriochrome Cyanine R staining was performed on spinal cord sections, as previously described,[11] for visualization. Dried sections were cleared in xylene, rehydrated in a reverse ethanol series followed by distilled water (dH_2O), then left in a solution containing 0.16% Eriochrome Cyanine R, 0.4% sulphuric acid, and 0.4% iron chloride (in dH_2O) to stain myelinated fibers. Following staining, sections were differentiated in 0.5% ammonium hydroxide and counterstained in neutral red, then rinsed in dH_2O. Lastly, sections were dehydrated and cleared, then mounted onto silane-coated SuperFrost™ Plus slides (Fisher Scientific, Pittsburgh, PA, USA). Pictures were taken of sections at 800-μm intervals throughout the lesion site (Zeiss AxioImager M2 microscope) to measure the distribution of hemorrhage (in percentage of the cross-sectional area). Images were analyzed using Zen Imaging Software (Carl Zeiss Canada Ltd., Toronto, Ontario, Canada).

Data analysis and statistical analysis

Comparisons between each treatment group for SCBF, PO_2, SCP, and metabolic responses were calculated using repeated measures analysis of variance (ANOVA) followed by Tukey's multiple comparisons test (GraphPad Prism 7). If more than two consecutive points in the elapsing time were statistically significant at $p < 0.05$, differences between treatment groups were considered significant. Results were plotted as mean values ± standard error of the mean (SEM) where values were collected at 1-min intervals for SCBF, PO_2, and SCP measurements, and 15-min intervals for microdialysis measurements. *F* values were reported as a group by time interaction unless stated otherwise. Pearson correlation coefficients (unadjusted for multiple comparisons) were calculated between 2-mm SCBF and PO_2 measurements.

Data for the hemorrhage analysis were plotted as mean values ± SEM for different positions (spaced 800 μm apart) along the length of the spinal cord (total 2.7 cm). The area under the curve (AUC) of total hemorrhage (%) was calculated to measure the cumulative amount of hemorrhage. Comparisons between each treatment group were calculated using a one-way ANOVA followed by Tukey's multiple comparisons test.

Due to the need for appropriate titration of NE dosages throughout the experiment, the surgery team was not blinded to the intervention groups. However, the research team was blinded for the data analysis of the physiological, metabolic, and histological measurements.

Results

Injury biomechanics

Impact force was measured, and displacement and velocity were calculated for each animal to evaluate the consistency of the injury (see Supplementary Table S1). No statistically significant differences were observed among the treatment groups for these parameters. Across all four experimental groups, the average maximum impact force measured from the tip of the impactor was 3296.5 ± 67.9 kdyn (mean ± SEM). The average cord displacement was 5.2 ± 0.1 mm with a velocity of 1836.5 ± 6.4 mm/sec at impact.

Hemodynamic outcomes

For the period of MAP augmentation, we elevated the MAP by ~20 mm Hg above baseline levels using NE. The target MAP was maintained by increasing or decreasing the NE infusion rate while the overall IV fluid replacement rates were kept constant. The average NE doses for the CP, DCP, and CP-DCP groups were respectively 26.4 ± 11.2 (range 11.4–81.7), 18.0 ± 5.6 (range 5.7–44.1), and 21.2 ± 6.7 (range 7.2–47.8) μg/kg/min. No significant differences were observed in target MAP values across the three treatment groups (CP: 82 mm Hg; DCP: 83 mm Hg; CP-DCP: 88 mm Hg) (Supplementary Fig. S2A). The NE infusion was associated with an increase in heart rate in all three groups, which declined when NE infusion was ceased. Interestingly, the increase in heart rate was significantly lower in the CP-DCP group compared with the CP group ($F[267, 1780] = 1.671$, $p < 0.05$) and DCP group ($F[360, 2400] = 8.272$, $p < 0.05$) (Supplementary Fig. S2B).

Post-injury changes in SCBF, PO_2, and SCP at the proximal measurement site (2 mm probe position)

At the proximal measurement site (2-mm position), SCBF immediately dropped below baseline values following SCI in all experimental groups (Fig. 1). NE infusion resulted in a prominent rise in SCBF of approximately 1.5- and 2.0-fold above baseline in the CP and DCP groups, respectively. Notably, however, the SCBF variability within each group was relatively high. The CP-DCP group also showed a related increase in SCBF upon NE infusion, although the effect was dampened compared with the CP and DCP groups.

PO_2 measurements reflected similar results as the SCBF data (Fig. 2). PO_2 significantly correlated with SCBF values in the control ($p < 0.0001$, r = 0.79), DCP ($p < 0.0001$, r = 0.80), and CP-DCP ($p < 0.0001$, r = 0.76) groups. After SCI and during

compression in all groups, PO_2 dropped 30–40 mm Hg below baseline values. Whereas the SCBF seemed to recover during the subsequent 30 min, PO_2 levels remained relatively flat and did not demonstrate any recovery during this period. Upon NE infusion, PO_2 levels in the CP and DCP groups significantly increased compared with the control group and increased to levels close to baseline values. Similarly, an augmented PO_2 response was observed in the CP-DCP group during the compressed and decompressed state of the injured spinal cord. However, this rise in PO_2 was not as large as in the CP group, and the values remained at approximately –20 mm Hg below baseline for the duration of the experiment.

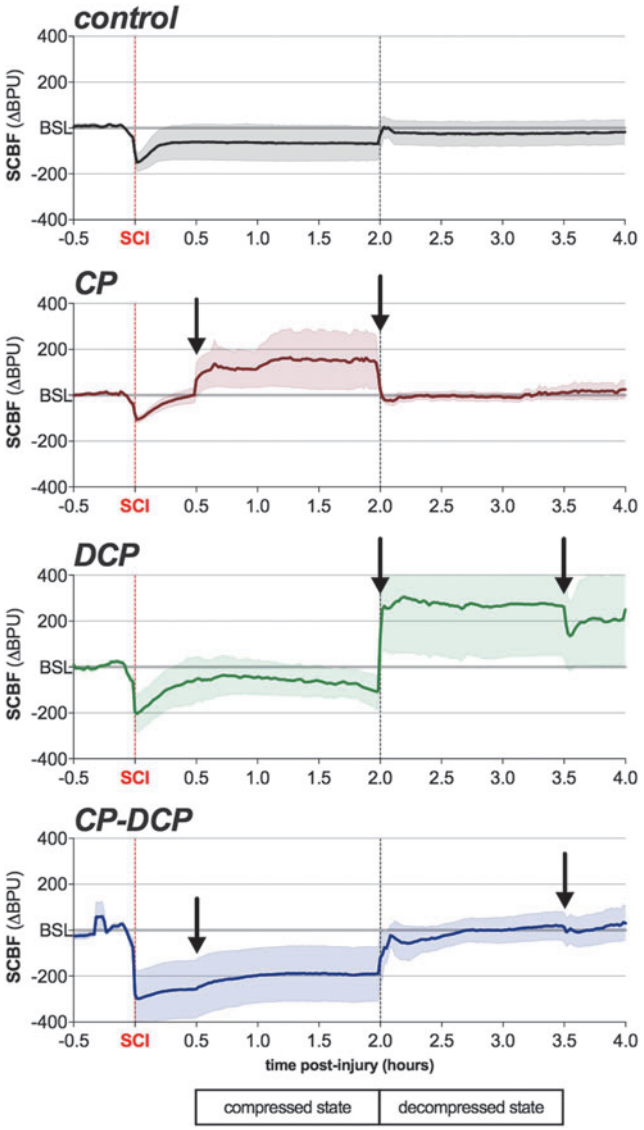

FIG. 1. Effect of MAP augmentation on SCBF (2-mm location). Intraparenchymal SCBF changes with MAP augmentation during the compressed (CP group), decompressed (DCP group), and both states (CP-DCP group) of the injured spinal cord. There was a modest recovery of SCBF in the CP, DCP, and CP-DCP group with MAP augmentation. Black arrows indicate the start and end of vasopressor infusion to increase MAP by ~20 mm Hg. Data are expressed as mean ± SEM. MAP, mean arterial blood pressure; SCBF, spinal cord blood flow; SEM, standard error of the mean. Color image is available online.

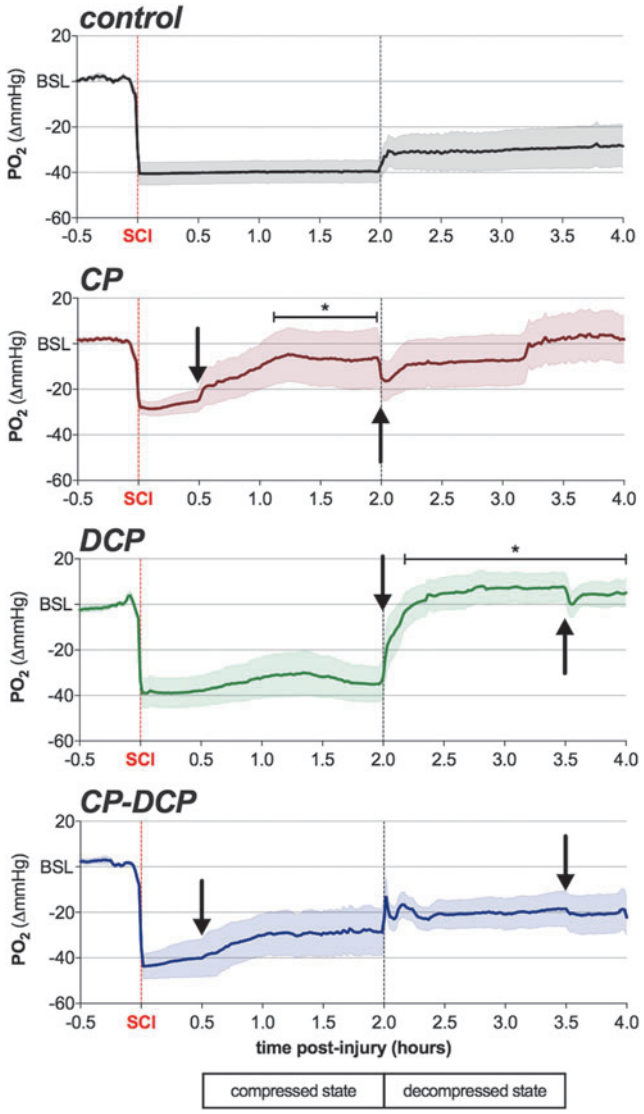

FIG. 2. Effect of MAP augmentation on spinal cord oxygenation (2-mm location). PO_2 responses of 1.5 h of NE infusion during the compressed (CP group), decompressed (DCP group), and both states (CP-DCP group) of the injured spinal cord. Between groups, NE infusion resulted in significantly higher PO_2 levels in the CP group ($F[267, 1780] = 1.707$, $^*p < 0.05$) and DCP group ($F[360, 2400] = 2.348$, $^*p < 0.05$) compared with the control group. The CP-DCP group showed a similar increase during compression; however, after decompression, NE infusion did not seem to affect the overall response in this group. Black arrows indicate the start and end of vasopressor infusion to increase MAP by ~20 mm Hg. Data are expressed as mean ± SEM. MAP, mean arterial blood pressure; NE, norepinephrine; PO_2, partial pressure of oxygenation; SEM, standard error of the mean. Color image is available online.

As expected, SCP increased after SCI and during compression; however, the extent of the increase was quite variable between groups/animals (Fig. 3). SCP appeared to increase upon NE infusion in the CP group; however, due to large intra-group variability, this rise in SCP was not statistically significant. Upon decompression, SCP values recovered to baseline levels within minutes in all groups and remained unchanged throughout the remainder of the post-injury period in the control, CP, and DCP group. Notably, in the CP-

DCP group (which received MAP augmentation both in the compressed and decompressed state), the SCP increased gradually post-decompression (~5 mm Hg) over the course of 2 h of observation. This gradual rise in SCP was not seen in the CP or DCP groups.

Post-injury metabolic responses at the proximal measurement site (2-mm probe position)

In all groups, GLUC levels dropped dramatically over the first 30 min after the contusion/compression injury (Fig. 4). In the control group, GLUC levels continued to gradually decrease for the next 1.5 h of compression. In contrast, with the initiation of MAP augmentation, GLUC levels increased in the CP group and held steady in the CP-DCP group. After decompression, significantly higher GLUC levels were observed in the CP-DCP group compared with the control group, whereas increased GLUC levels were also observed in the CP and DCP groups although they were not statistically significant. The average GLUC values for the groups receiving MAP augmentation were close to or above baseline, as

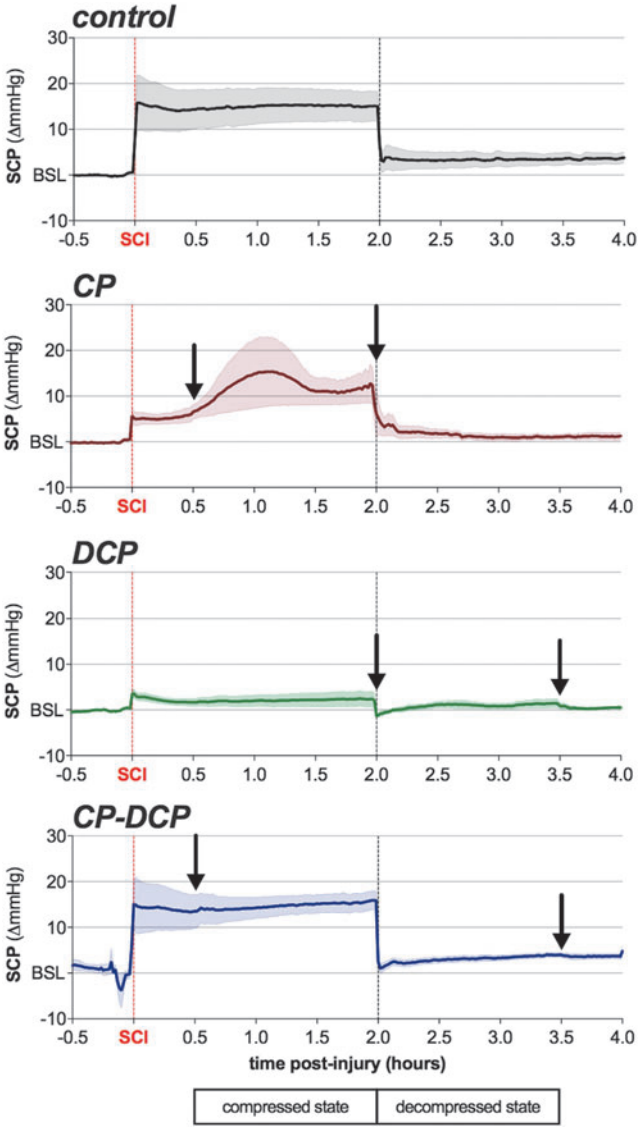

FIG. 3. Effect of MAP augmentation on spinal cord pressure (2-mm location). SCP responses of 1.5 h of NE infusion during the compressed (CP group), decompressed (DCP group), and both states (CP-DCP group) of the injured spinal cord. NE infusion following decompression resulted in a small but steady increase in SCP in the CP-DCP group. Black arrows indicate the start and end of vasopressor infusion to increase MAP by ~20 mm Hg. Data are expressed as mean ± SEM. MAP, mean arterial blood pressure; NE, norepinephrine; SCP, spinal cord pressure; SEM, standard error of the mean. Color image is available online.

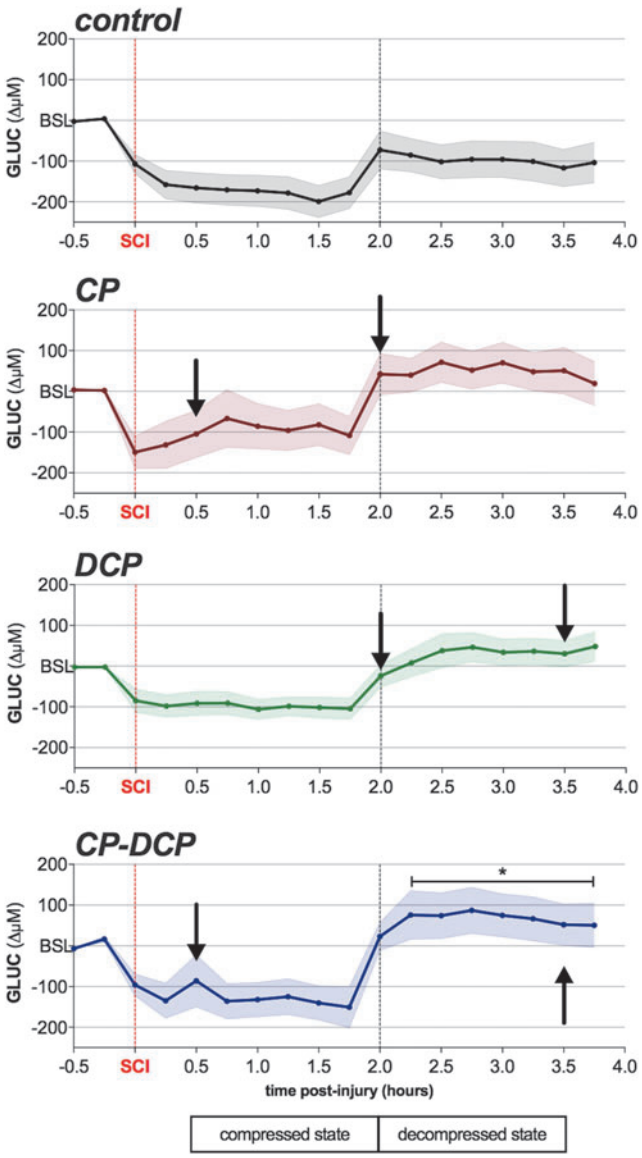

FIG. 4. Effect of MAP augmentation on glucose levels (2mm location). GLUC response of 1.5 h of MAP augmentation during the compressed (CP group), decompressed (DCP group), and both states (CP-DCP group) of the injured spinal cord. Contrary to the gradual drop in GLUC for the control group during the 1.5 h of compression, GLUC levels during NE infusion increased in the CP group and remained steady in the CP-DCP group. After decompression, GLUC levels were significantly higher in the CP-DCP group ($F([18, 120] = 1.963$, $^*p < 0.05$) compared with the control group. Black arrows indicate the start and end of vasopressor infusion to increase MAP by ~20 mm Hg. Data are expressed as mean ± SEM. GLUC, glucose; MAP, mean arterial blood pressure; NE, norepinephrine; SEM, standard error of the mean. Color image is available online.

compared with the control group whose values remained below baseline (about −100 μM from baseline).

The spinal cord contusion/compression injury resulted in an immediate increase in the L/P ratio in all four experimental groups (Fig. 5). Upon the initiation of NE infusion in both the CP and CP-DCP groups, the L/P ratio decreased. During this same period of time, the L/P ratio remained stable in the control and DCP groups. After decompression, NE infusion did not seem to affect the L/P ratio, and all groups showed a decline toward baseline values.

NE infusion did not appear to influence the SCI-induced rise in GLUT or decompression-induced rise in GLY levels as observed in the CP, DCP, and CP-DCP groups (Fig. 6).

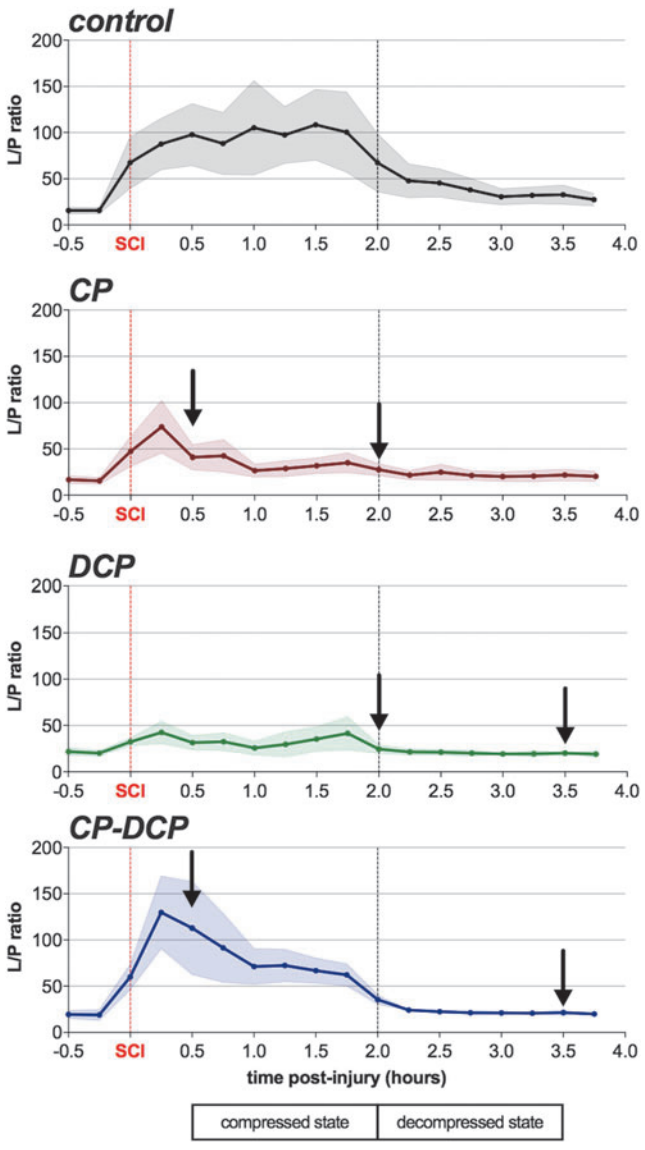

FIG. 5. Effect of MAP augmentation on L/P ratio levels (2-mm location). L/P ratio response of 1.5 h of MAP augmentation during the compressed (CP group), decompressed (DCP group), and both states (CP-DCP group) of the injured spinal cord. NE infusion during the compressed state decreased the L/P ratio in both the CP and CP-DCP group. Black arrows indicate the start and end of vasopressor infusion to increase MAP by ~20 mm Hg. Data are expressed as mean ± SEM. L/P, lactate/pyruvate; MAP, mean arterial blood pressure; SEM, standard error of the mean. Color image is available online.

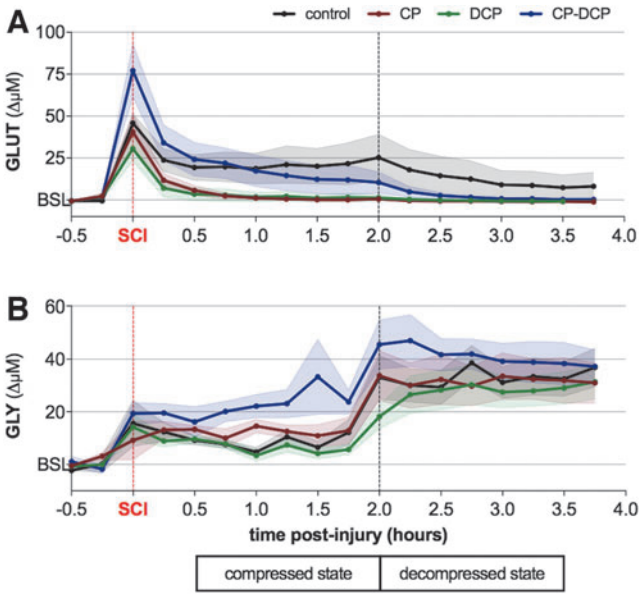

FIG. 6. Effect of MAP augmentation by 20 mmHg using NE after SCI on GLUT and GLY levels (2-mm location). **(A)** GLUT and **(B)** GLY responses of 1.5 h of MAP augmentation during the compressed (CP group), decompressed (DCP group), and both states (CP-DCP group) of the injured spinal cord. NE infusion did not have a noticeable effect on GLUT or GLY levels. Data are expressed as mean ± SEM. GLUT, glutamate; GLY, glycerole; MAP, mean arterial blood pressure; NE, norepinephrine; SCI, spinal cord injury; SEM, standard error of the mean. Color image is available online.

Post-injury changes in SCBF, PO_2, and SCP at the distal measurement site (22-mm probe position)

Compared with the 2-mm probe location, slight changes in SCBF, PO_2, and SCP after SCI were observed at the 22-mm position (i.e., the more distal of the two probe positions (Fig. 7). NE infusion demonstrated no noticeable effect on SCBF, PO_2, or SCP at this measurement site, further away from the SCI ''penumbra'' than the 2-mm probe.

Post-injury metabolic responses at the distal measurement site (22-mm probe position)

At the 22-mm position, GLUC, GLUT, GLY, and L/P ratio responses after SCI were far less pronounced than that observed at the 2-mm position (Fig. 8). Except for GLUC, no significant differences were observed among the experimental groups. Similar to the 2-mm location, NE infusion significantly increased GLUC levels in the CP-DCP group compared with the control group (Fig. 9). Increased GLUC levels were also observed in the CP and DCP groups, although these changes were not statistically significant.

Spinal cord tissue hemorrhage

Hemorrhage was observed within the spinal cord in all animals acutely following contusive/compression injury at and near the site of injury (Fig. 10). The CP-DCP group, which received the longest period of NE infusion, demonstrated greater hemorrhage at and up to 5 mm rostral to the epicenter when compared with the CP, DCP, and control group. On average, about one fourth of the cross-sectional area (25%) was occupied by hemorrhage at the epicenter

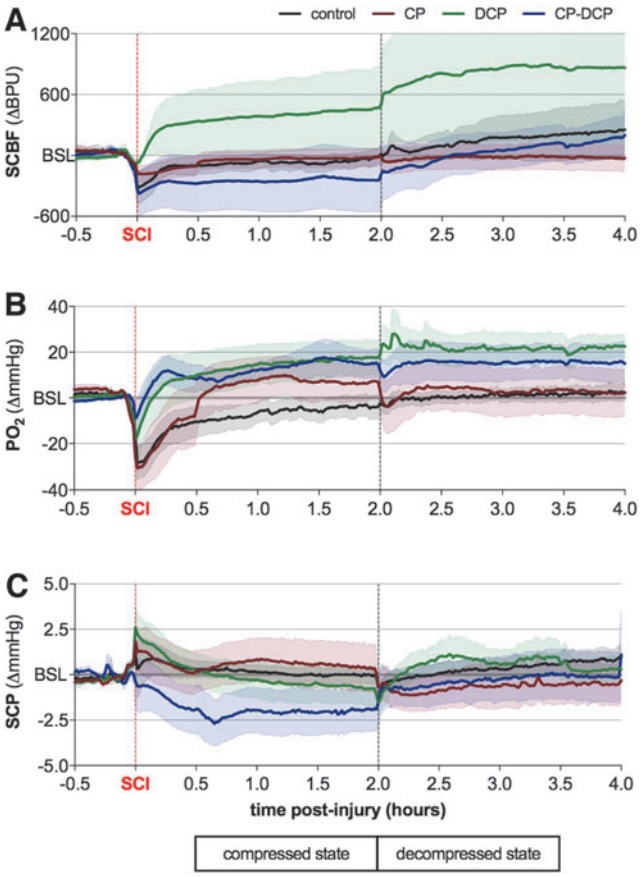

FIG. 7. Effect of MAP augmentation by 20 mm Hg using NE after SCI on intraparenchymal blood flow, oxygenation and pressure (22 mm location). **(A)** SCBF, **(B)** PO_2, and **(C)** SCP responses of 1.5 h of NE infusion during the compressed (CP group), decompressed (DCP group), and both states (CP-DCP group) of the injured spinal cord. More distal to the injury, NE infusion did not have a noticeable effect on SCBF, PO_2 or SCP. Data are expressed as mean ± SEM. MAP, mean arterial blood pressure; NE, norepinephrine; PO_2, partial pressure of oxygenation; SCI, spinal cord injury; SCP, spinal cord pressure; SEM, standard error of the mean; SCBF, spinal cord blood flow. Color image is available online.

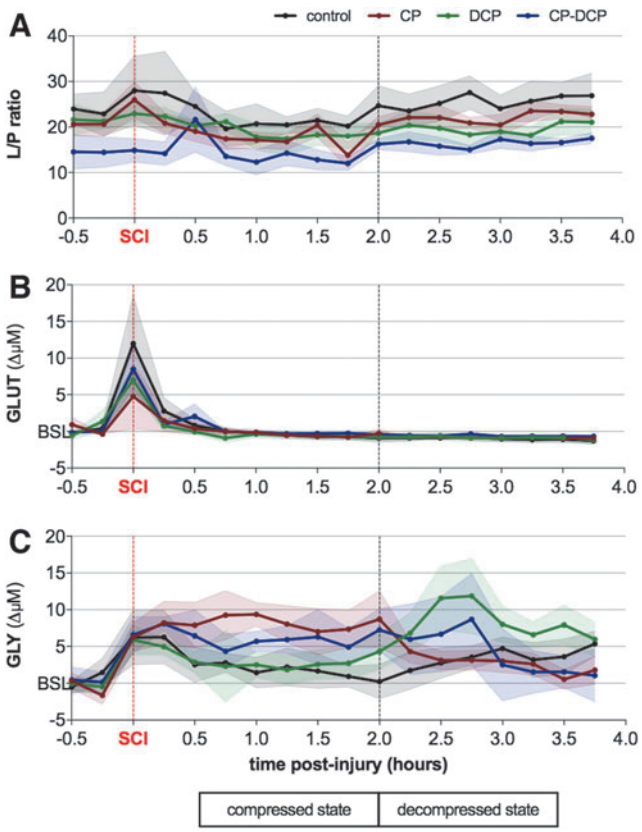

FIG. 8. Effect of MAP augmentation by 20 mm Hg using NE after SCI on the L/P ratio, GLUT and GLY levels (22-mm location). **(A)** L/P ratio, **(B)** GLUT, and **(C)** GLY responses of 1.5 h of NE infusion during the compressed (CP group), decompressed (DCP group), and both states (CP-DCP group) of the injured spinal cord. More distal to the injury, NE infusion did not have a noticeable effect on L/P ratio, GLUT or GLY levels. Data are expressed as mean ± SEM. GLUT, glutamate; GLY, glycerole; L/P, lactate/pyruvate; MAP, mean arterial blood pressure; NE, norepinephrine; SCI, spinal cord injury; SEM, standard error of the mean. Color image is available online.

in the CP-DCP group, versus 14%, 16%, and 17% in the CP, DCP, and control groups, respectively. Similarly, there was significantly greater cumulative hemorrhage in the CP-DCP group, calculated as the AUC for the calculated hemorrhage measurements from all sections. No significant differences were observed between the CP, DCP, and control groups.

Discussion

Hemodynamic management is one of the few treatment options to potentially improve neurological recovery of individuals who sustain an acute SCI. Current clinical guidelines recommend elevating MAP to 85–90 mm Hg using IV fluids and vasopressors for 7 days following acute SCI.[7] Although improved outcomes have been observed from aggressive hemodynamic support,[3–6] several studies have also found no benefit in neurological recovery.[17–20] Examining the factors that may contribute to the difficulty in convincingly demonstrating the relationship between MAP augmentation and improved neurological recovery is warranted. It is important to consider that the approach of augmenting MAP with vasopressors may be providing not only beneficial but also adverse effects on the injured spinal cord. We undertook this study to characterize the effect of MAP augmentation on the injured spinal cord when it was in its compressed and decompressed state. An understanding of how MAP augmentation affects the acute vascular and metabolic responses of the spinal cord in these two conditions is important for optimizing the hemodynamic management of SCI. To our knowledge, this represents the first comprehensive study of combined IP physiological and metabolic responses comparing MAP augmentation during the injury phase of sustained compression and the period after decompression of the spinal cord.

At the proximal measurement site, MAP augmentation during compression increased SCBF levels ~174% of pre-injury values in the CP group, whereas MAP augmentation during compression failed to increase SCBF above baseline in the CP-DCP group. This was unexpected, given that these two groups are in fact the same immediately after the contusive SCI when sustained compression is applied, and they differ only after the decompression is performed. Although there were similar target MAP levels in both

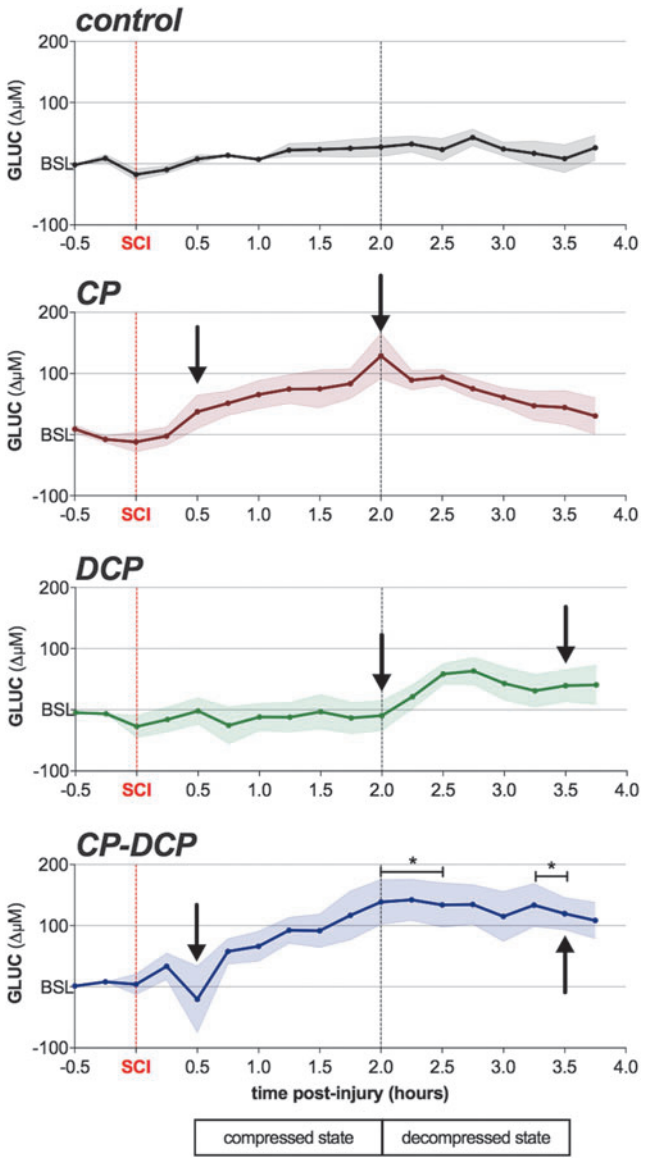

FIG. 9. Effect of MAP augmentation on glucose levels (22-mm location). GLUC response of 1.5 h of MAP augmentation during the compressed (CP group), decompressed (DCP group), and both states (CP-DCP group) of the injured spinal cord. More distal to the epicenter, NE infusion significantly increased GLUC levels in the CP-DCP group ($F[18,120] = 2.207$, $^*p < 0.05$) compared with the control group. Black arrows indicate the start and end of vasopressor infusion to increase MAP by ~20 mm Hg. Data are expressed as mean ± SEM. GLUC, glucose; MAP, mean arterial blood pressure; NE, norepinephrine; SEM, standard error of the mean. Color image is available online.

groups, NE significantly increased heart rate in the CP group (Supplementary Fig. S2B). The greater cardiac output associated with the higher heart rate in the CP group compared with the CP-DCP group may explain differences in SCBF recovery with MAP augmentation. Previous studies have also observed increased heart rate[11] and increased cardiac output,[21] the product of heart rate and stroke volume, in animals with improved SCBF. Although speculative, it is intriguing to consider the possibility that IP blood flow around the injury site may be influenced in this manner by cardiac output/function. This is the subject of ongoing study in our lab.

Similarly, MAP augmentation during only the decompressed state of injury restored SCBF levels ~200% of pre-injury values in the DCP group, whereas a minimal increase above baseline was observed in the CP-DCP group. Again, this could similarly be due to the significantly higher heart rate and increased cardiac output in the DCP group compared with the CP-DCP group (Supplementary Fig. S2B), although we note that large intra-group variability was also observed in the DCP group. MAP augmentation in the CP-DCP group increased SCBF, but due to a lower heart rate and large variability among animals, the increase in SCBF was dampened compared with the CP and DCP groups. Previous studies reported increased SCBF upon vasopressor administration in both a lamb[22] and porcine[11] model of SCI.

SCBF preservation is critical immediately following SCI to limit ischemia and subsequent secondary injury. Experimental studies have shown that reduction in SCBF after spinal cord compression significantly impaired neurological recovery after SCI,[23] which correlated with dysfunction in axonal conduction in motor and somatosensory spinal tracts,[24] whereas increased SCBF was associated with improved axonal function after acute SCI.[25] Our results suggest that MAP augmentation during the compressed, decompressed, and throughout both states of injury increases SCBF and consequently microvascular tissue perfusion proximal to the site of injury, whereas differences in heart rate may be associated with sufficient alterations in cardiac output to impact the magnitude of the increase.

Further, MAP augmentation during only compression or only post-decompression appeared to significantly increase PO_2 levels close to or above pre-injury values proximal to the site of injury and demonstrate an increasing trend distal to the site of injury. MAP augmentation during both states of injury also increased PO_2 levels in the CP-DCP group; however, the increase in PO_2 was dampened compared with the CP and DCP groups, resembling SCBF measurements. Overall, PO_2 measurements similarly reflect the changes observed in SCBF.

Previous work in a canine model of SCI demonstrated that increased perfusion in spinal cord-feeding arteries improved intrathecal PO_2 and protected against post-traumatic ischemia.[26] This suggests that MAP augmentation increases cellular oxygen availability proximal to the site of injury to prevent ischemic injury. With respect to tissue hemodynamics directly adjacent to the site of injury, our data suggest that MAP augmentation during the compressed, decompressed, or continuously throughout both states of injury, modestly improves IP blood flow and oxygenation.

In addition to direct measurements of tissue hemodynamics, we were also interested in examining the effect of MAP augmentation on downstream metabolic responses by measuring metabolic analytes via microdialysis. Previous work suggests monitoring LAC and PYR levels as markers of tissue perfusion and tissue oxygen metabolism status, in which an elevated L/P ratio was associated with decreased blood flow to the cord in both a primate model[27,28] and feline model of SCI.[29,30] In such ischemic/hypoxic environments, anaerobic glycolysis occurs to transform GLUC to LAC and convert PYR to LAC, which ultimately increases the L/P ratio. An increased L/P ratio was previously observed to reflect prolonged tissue hypoxia at the injury site following contusion-compression SCI.[31]

Our results reveal a decreasing trend in the L/P ratio with MAP augmentation during spinal cord compression, suggesting a reduction of ischemia and hypoxia in the cellular environment adjacent to the site of injury. We also observed an increasing trend in GLUC at both the proximal and distal measurement sites upon MAP augmentation during both injury states in the CP-DCP

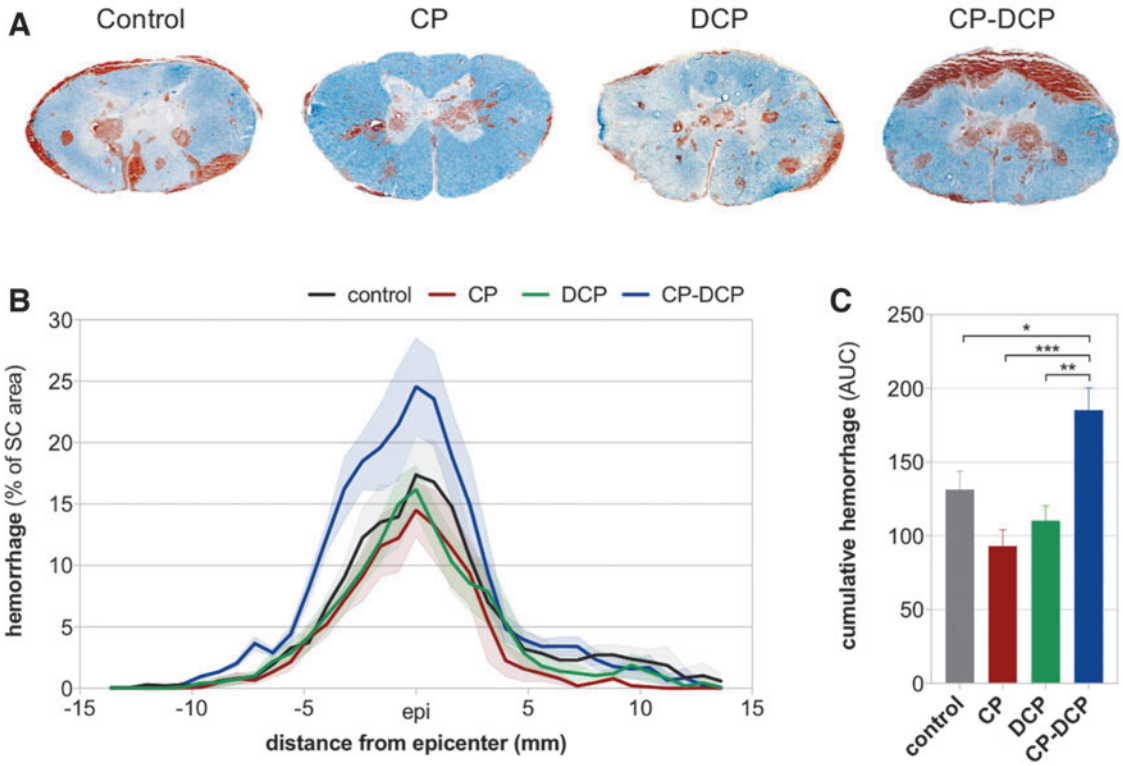

FIG. 10. Effect of MAP augmentation by 20 mm Hg using NE after SCI on spinal cord hemorrhage. **(A)** Representative images from Eriochrome Cyanine stained axial sections of the spinal cord from the control, compressed (CP), decompressed (DCP), and CP-DCP groups at the epicenter of injury. **(B)** The total hemorrhage calculated as the percentage of the spinal cord area of each axial section. The CP-DCP group demonstrated the greatest average hemorrhage at the epicenter of injury (~25%). **(C)** The total volume of hemorrhage (cumulative hemorrhage) calculated as the area under the curve of total hemorrhage (%) was significantly increased in the CP-DCP group compared with the CP group ($F[3, 20] = 10.43$, $^{***}p = 0.0002$), DCP group ($F[3, 20] = 10.43$, $^{**}p = 0.002$), and control group ($F[3, 20] = 10.43$, $^{*}p = 0.03$) (ANOVA). Data are expressed as mean ± SEM. ANOVA, analysis of variance; MAP, mean arterial blood pressure; NE, norepinephrine; SCI, spinal cord injury; SEM, standard error of the mean. Color image is available online.

group. A recent study monitoring spinal cord perfusion pressure (SCPP) and microdialysis directly at the site of injury in human acute SCI patients found that an increased SCPP of 90–100 mm Hg resulted in lower L/P ratios and higher GLUC levels.[32] This may explain why MAP augmentation (which increases SCPP) during cord compression resulted in a reduction of the L/P ratio at the proximal measurement site (2 mm).

Our findings of significantly larger IP hemorrhage in animals receiving continuous NE infusion during both the compression and post-decompression phase of injury are particularly interesting. Based on studies examining intracerebral hemorrhage, acute increases in blood pressure have been associated with increased hemorrhage.[33] Acutely elevated blood pressure increases systemic vascular resistance,[34] contributing to the rupture of cerebral blood vessels.[35] Others have also found that increasing blood pressure elevated levels of superoxide in blood vessels,[36] which increases oxidative stress. This increased oxidative stress induces the cell death of endothelium and vascular muscle,[37,38] impairing blood vessels and ultimately increasing cerebral hemorrhage.[39] These findings are relevant to the traumatically injured spinal cord, where the microvasculature of the spinal cord has been shown to be weakened and compromised following injury.[40] In a T10 rat model of SCI, increased IP hemorrhage was also observed after NE infusion for MAP augmentation post-injury.[41] The authors started NE infusion 15 min after injury and already observed increased hemorrhage size 60 min post-injury. Another study in a rat model of SCI found that animals receiving epinephrine to induce hypertension following injury demonstrated hyperemia in the injured cord, which could potentially lead to increased hemorrhage and edema.[8]

These results represent both an important and concerning finding: that aggressive hemodynamic support following acute SCI can lead to parenchymal bleeding within the injured spinal cord. With efforts to improve blood flow and reduce ischemia in the injured cord by augmenting MAP via vasopressors, clinicians may inadvertently promote undesirable bleeding within the spinal cord, thereby increasing the size of IP hemorrhage at the injury site. In a rat model of SCI, increased areas of hemorrhage were correlated with greater functional deficits.[42,43]

In addition, studies using magnetic resonance imaging (MRI) in human patients with SCI found that a greater degree of hemorrhage was associated with poorer neurological outcomes.[44] Although this may simply reflect the severity of the initial mechanical injury, blood products themselves within the injured cord parenchyma may be detrimental for recovery. Specifically, the degradation of free heme from hemoglobin released via red blood cell lysis produces excess levels of free iron, which have been shown to be highly toxic in neural tissue[45–48] and lead to progressive neuronal and glial loss.[49,50] In fact, the accumulation of iron can generate free radicals and oxidative damage,[48] and has been found to increase tumor necrosis factor (TNF) expression and promote pro-inflammatory M1 macrophage polarization, a detrimental macrophage state for

recovery.[51] Our demonstration of increased IP hemorrhage after continuous NE in both the presence and absence of cord compression may contribute to the explanation of why contrasting findings in neurological outcome following MAP management have been reported. To date, we are unaware of a clinical study that has evaluated the use of vasopressors and hemorrhage within the cord after human SCI.

We also observed SCP gradually increase post-decompression in animals with elevated MAP during both the compressed and decompressed state of injury. This gradual rise in SCP was not seen in the CP or DCP groups. In human SCI patients, increased intraspinal pressure (ISP) was shown to reduce perfusion to the spinal cord, as represented by SCPP, which can therefore worsen post-traumatic ischemia.[52] A previous study in a T10 rabbit model of SCI found that increased spinal cord hemorrhage drives increased ISP initially after traumatic SCI.[53] The increased hemorrhage observed in the CP-DCP group may explain the gradual increase of SCP observed in this group. This suggests that continuous MAP augmentation throughout the compressed and decompressed state is not only increasing IP hemorrhage but may also unintentionally increase hydrostatic pressure within the spinal cord.

Our data highlight the elegant proposal by Saadoun and Papadopoulos[54] that perhaps optimizing SCPP may be more important than optimizing MAP (with SCPP calculated as the difference between MAP and ISP). This suggestion is analogous to management in traumatic brain injury (TBI) where instead of ISP, intracranial pressure (ICP) is measured along with MAP to monitor and optimize cerebral perfusion pressure (CPP).[55–57] The concept of using SCPP to guide hemodynamic management is still evolving. A recent prospective observational study found that SCPP as measured with a lumbar intrathecal catheter can better predict neurological recovery following SCI, where cutoff values for SCPP were a more robust marker than cutoff values for MAP in predicting poor neurological outcome.[58] Although we acknowledge that measuring SCPP with a pressure probe at the site of injury may differ from using a pressure probe in the lumbar cistern, these data support the principle that managing SCPP may be favorable over managing MAP for promoting neurological recovery following SCI.

In this study, we employed a porcine model of SCI as it offers several advantages as a large animal model. The vascular supply and cardiovascular physiology in the porcine spinal cord are strikingly comparable to the human spinal cord, and physiological parameters such as cardiac output and vascular distribution share resemblance.[59] In addition, studies have shown that even small segmental thoracic and lumbar arteries[60] and fine vessels in the subarachnoid space are parallel between pigs and humans.[61] Porcine models have therefore been extensively used to model several cardiovascular diseases and spinal vascular pathologies in published studies.[62] Together, these anatomical and physiological similarities between pigs and humans make the pig a clinically relevant model for studying the effect of MAP augmentation on IP physiological and metabolic changes following SCI.

Lastly, we recognize there are limitations in this study that are worth discussing. As in humans, variabilities are observed in normal MAP measurements in healthy awake pigs, ranging from 85 to 113 mm Hg.[63–65] In this current study, the combination of anesthesia and a traumatic SCI results in a decreased MAP of about 60–80 mm Hg and increasing MAP by 20 mm Hg actually brings MAP up to normotensive levels at best. MAP augmentation may perhaps be at hypotensive levels for some animals and not hypertensive (which in fact may also be true in human SCI). In addition, we recognize this acute investigation is not a completely accurate representation of the clinical environment in which patients are receiving vasopressors and a plethora of other drugs for many hours to days post-injury. Patients may also have compression of the cord for many hours to days, whereas our experiment included only 2 h of compression and 2 h of post-decompression monitoring. This limited time frame for investigation was related to the lengthy experimental procedure that often started at 6:00 a.m. and ended late in the evening. Future studies can investigate the direct interaction between vasopressors and the combination of drugs patients are receiving after injury, along with longer periods of observation.

Moreover, despite precise insertion of the IP probes into the spinal cord at exact distances from the injury site, we observed variability in both pre- and post-injury measures. One potential reason for this variability is subtle differences in the position and depth of the probe tip with respect to the gray and white matter, and thus their relative proximity to vascular supply.[66] Despite these limitations, given our brief period of MAP augmentation, increased IP hemorrhaging following 3 h of NE infusion warrants consideration.

This study provides a comprehensive description about the acute physiological and metabolic responses following the hemodynamic management of MAP after traumatic SCI. Our data suggest that MAP augmentation during compression and post-decompression, and continuously throughout both injury states, modestly restores IP blood flow and oxygenation, whereas spinal cord pressure appears to steadily rise with MAP augmentation during both injury states. Elevating MAP during cord compression appears to reduce the L/P ratio, reflecting a lesser degree of ischemia in the injured cord. Most importantly, our findings suggest that continuous MAP augmentation during the compressed and decompressed state of injury may be detrimental due to increased cumulative hemorrhage near the injury site. Overall, we hope our findings can inform clinicians on the optimal timing and efficacy of MAP augmentation after SCI to optimize the hemodynamic management of patients with acute SCI and ultimately improve neurological recovery for patients with SCI.

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Author Disclosure Statement

No competing financial interests exist.

Supplementary Material

Supplementary Figure S1
Supplementary Figure S2
Supplementary Table S1

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Address correspondence to:
Brian K. Kwon, MD, PhD, FRCSC
Department of Orthopedics
University of British Columbia
Blusson Spinal Cord Center, 6th Floor
Vancouver General Hospital
818 West 10th Avenue
Vancouver, British Columbia V5Z 1M9
Canada

E-mail: brian.kwon@ubc.ca